USGS

WATER RESOURCES RESEARCH GRANT PROPOSAL

Title: The Assessment of Fecal Indicator Potential and Degradation Kinetics of Caffeine and Several Caffeine Metabolites in Environmental Waters

Focus Category: TS, WQL, NC, MET

Keyword Numbers: Groundwater Modeling, Groundwater Management, Groundwater Movement, Hydrogeology, Karst Hydrology, Rainfall, Fecal Indicators, Tropical Waters

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Congressional District of University Performing Research: N/A

Statement of the Critical State or Regional Water Problem

Until recently pharmacologically active substances which have entered into the environment have received little attention. These compounds are uniquely and unambiguously associated with human activity, especially human waste. It is with regard to the absolute anthropogenic association of caffeine in the environment that we propose the present project.

Infectious agents that are present in recreational waters pose a significant health risk to people using those waters. Human pathogens may enter recreational waters from a variety of sources. Human fecal waste potentially contains a wide variety of pathogenic organisms that can cause illness and disease in humans. The greatest health risk involves contact with recreational water that has been polluted with human fecal waste. As the population base that the fecal waste is derived from increases in size, the potential for risk increases as well, both in terms of the types and numbers of pathogens present. It is generally agreed that the public should be warned if recreational waters present a health risk.

Since 1972 the US Environmental Protection Agency (US EPA) has required analysis of recreational waters for indicator organisms in an attempt to define the risk of waterborne and water contact disease to the public. Originally that indicator standard was Fecal Coliform bacteria. These are the coliform bacteria that commonly occur in the human gut

and feces. Since 1978 the Guam Environmental Protection Agency (GEPA) has analyzed water for indicator bacteria. Originally, a health advisory was issued for those recreational areas with fecal coliform counts in excess of 200 colony forming units (CFU) per 100 mL. In 1986 this standard was changed to reflect decreased confidence in the Fecal Coliform test in representing disease-associated risk. Currently US EPA mandates and GEPA uses the Enterococcus Test for the evaluation of marine waters and *Escherichia coli* (*E. coli*) for evaluation of recreational fresh waters. Enterococcus is a subgroup of the fecal streptococci and *E. coli* is one species of the fecal coliform group. It has been determined, at least in temperate waters, that the presence of significant numbers of either of these two groups indicates an increased risk of waterborne and/or water contact disease. The current standard for marine waters is 35 cfu of enterococcus / 100 mL. The fresh water standard is 126 cfu *E. coli* / 100 mL. It should be noted that GEPA does not use the preferred enterococcus test in fresh waters due to the inability to meet the mandated standard because of high counts of enteroccus.

In recent years, even the utility of Enterococcus and *E. coli* as indicators of increased risk, especially in the tropics, has come into question (McNeil 1992). Both of these organisms have been shown to be able to live for prolonged times, and even multiply in tropical environments. The US EPA is currently seeking methods which more reliability correlate with increased risk of water associated disease. The proposed study will evaluate caffeine and caffeine metabolites as potential indicators of fecal pollution.

Statement of Results, Benefits, and/or Information Expected:

Currently, recreational waters are monitored on a weekly basis for federally mandated indicators of fecal pollution. In the case of marine waters that indicator is enterococcus. In the case of freshwaters that indicator is *E. coli*. Health advisories are made on the basis of the numbers of the respective indicator. It is clear that human fecal pollution is associated with increased risk of waterborne and water contact disease. Because there is concern that these indicators do not necessarily indicate risk in our tropical waters, the proposed study will evaluate the utility of caffeine and several caffeine metabolites for fecal indicator potential.

Caffeine is a compound that is present in several beverages, specifically coffee, tea and carbonated drinks. In addition it is present in pharmaceutical products to enhance analgesic action and provide central nervous stimulation. Caffeine is present in municipal waste waters of populations that use caffeine at levels up to 300 ug/L (Rogers, Birtwel and Kruzynski, 1986). Caffeine that is present in environmental water is predominately derived from human contributions to the sewage stream. Caffeine and its metabolites are excreted in the urine of individuals who have consumed beverages and pharmaceuticals containing caffeine. Additionally some of the caffeine metabolites are pharmaceutical agents (theophylline) or present in other beverages and foods (theobromine). Caffeine is known to be associated with the soluble fraction in water (Spectrum, 1998). Caffeine metabolites, however, have not been studied in this respect.

Results of this study will help elucidate the potential of using caffeine and its metabolites as an alternative indicator of fecal pollution. An evaluation of caffeine and/or its metabolites as indicators, in terms of technological feasibility, cost and rapidity of analysis will be provided.

Nature, Scope, and Objectives of the Research

The research is designed to:

A. Quantitate the presence of caffeine and its important metabolites in the sewage stream and in treated sewage. This would include temporal variations.

B. Study degradation rates of caffeine and its important metabolites under environmental conditions in raw and treated sewage and receiving waters.

C. Study the association of caffeine and its metabolites relative to the soluble vs particulate fractions. Sorption equilibria, particularly onto suspended solids will be evaluated.

Methods, Procedures and Facilities

Analysis will be based upon a solid-phase extraction - High Performance Liquid Chromatography (HPLC) method developed by the U.S. Geological Survey (Burkhardt et al, 1999). This method will be evaluated with respect to caffeine metabolites and modified as required. The US EPA has also published a method (US EPA, 1996) that will be evaluated for use and modification. The analysis of caffeine and its metabolites in clinical samples (urine and serum) are well-developed (Lu et al, 1998) and will be used as a starting point to modify the methods for environmental samples.

Studies will be conducted to understand the degradation kinetics of caffeine and its major metabolites in environmental samples. It is important to know the stability of the compounds under environmental conditions when considering a compound's indicator potential. A very labile compound may disappear from the environment before the infectious potential does. Further, various treatment processes may remove the compound. Various conditions, including microbiological flora and UV regime have major effects on the stability of chemicals in the environment.

Studies will also be conducted to understand the sorption characteristics of the metabolites onto suspended solids in sewage and receiving waters. It is important to understand sorption characteristics of a potential indicator chemical so as to know the type of sampling and sample preparation required.

All of the required instruments are currently in use at the WERI laboratory. WERI instruments and personnel will be utilized for the analysis of caffeine and its metabolites.

Related Research

There are numerous organisms from a variety of classes that can cause waterborne or water contact disease in humans. Some of these diseases are quite common, while other are uncommon or even rare. Some of the diseases are rather benign while others are quite serious. Common or rare, benign or serious, the combined effect of waterborne and water contact disease is very significant worldwide in terms of both morbidity and mortality. It is conservatively estimated that over one half of the world's population has suffered waterborne or water contact disease. Over 250 million new cases per year are reported, many more going unreported. This results in over 10 million deaths per year. At least 75% of these deaths are from tropical areas and 50% of the deaths occur in children less than five years old living in tropical areas. (World Health Organization (WHO), 1995). Over 940,000 episodes of illness are reported in the United States each year.

Since such a large number of pathogens are potentially present in recreational water, it is not practical to test water for all of them. This is so because the determination of some pathogens is very difficult and expensive. It is not technologically feasible to analyze for some pathogens in environmental samples. Even those pathogens that are readily and inexpensively determined singly, there are such a large number that it would be prohibitively expensive to assay for them all, or even the most likely ones. Therefore, a concept of analyzing for indicators of fecal pollution has become popular, as well as necessary. This concept is based upon the idea that the fecal – oral transmission route is the most important route for the transmission of human waterborne and water contact disease. Since the human gut contains a wide variety of non-pathogenic organisms, fecally derived sewage contains a large number of a wide variety of microorganisms, most of which are not pathogenic. An attempt has been made to assay for some of these organisms as indicators of fecal pollution.

Until recently the environmental presence of pharmacologically active substances has received little attention. These materials are normally at very low concentration in the environment and thus were always surmised to have little effect. However, these substances have been developed and used to attain a biological effect and may have an environmental effect out of proportion to their concentration. Additionally, these compounds are uniquely and unambiguously associated with human activity. (Halling-Sorensen et al, 1998).

Caffeine is both a naturally occurring and a commercially produced organic compound that is used in a variety of beverages and foods as well as drugs. Most notably, it is present in soft drinks and coffee. Since a portion of an administered dose is excreted unchanged in the urine it is present in sewage. Caffeine is reasonably soluble in water, in not volatile and appears not to be associated with sediments. It has been speculated that caffeine has promise as an indicator of human sewage pollution if the population being studied uses caffeine. No studies have been identified which have addressed this.

However, caffeine has been detected in domestic wastewater effluent, environmental surface water samples and finished drinking water in several locations worldwide (Raloff, 1998; Rogers et al, 1986 and Spectrum Laboratories 1998). However, there are only small data sets relative to the environmental concentrations of caffeine (Pereira et al, 1995 and Meade, 1995).

Caffeine undergoes a complex metabolism in the human. Predominant metabolites include paraxanthine (1,7-dimethyl xanthine), theobromine (3,7-dimehtyl xanthine) and theophylline (1,3-dimethyl xanthine), the three monomethyl xanthines and seven methylated uric acids. Only 3 percent of an administered caffeine dose is excreted unchanged making caffeine (Kashuba, 1999). This fact indicates that it may be a relatively insensitive indicator. The above mentioned metabolites will, therefore, be evaluated for indicator potential. Although caffeine has been demonstrated in environmental waters (Raloff, 1998; Rogers et al, 1986 and Spectrum Laboratories 1998) no published study has been identified which addresses the issue of the presence of caffeine metabolites in environmental waters.

This project will complement a study currently in progress:The Evaluation of Several Chemical Indicators of Fecal Pollution in Relationship to Standard Microbiological Indicators.

Progress Report: N/A

Literature Cited:

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